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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 February 2001 (08.02.2001)

PCT

(10) International Publication Number
WO 01/09604 A1

(51) International Patent Classification[7]: G01N 33/48

(21) International Application Number: PCT/US00/20646

(22) International Filing Date: 28 July 2000 (28.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/145,856 28 July 1999 (28.07.1999) US

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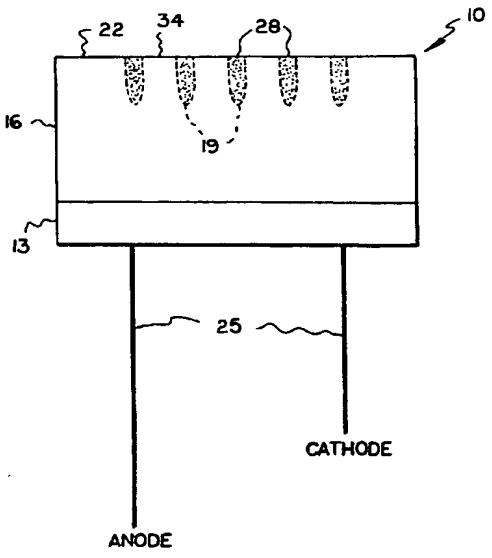
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MICROSENSOR ARRAYS AND METHOD OF USING SAME FOR DETECTING ANALYTES



(57) Abstract: A device for the detection of one or more analytes in a sample. The device comprises an electromagnetic radiation generator (13) having one or more chemical sensors (28) thereon.

WO 01/09604 A1

MICROSENSOR ARRAYS AND METHOD OF USING SAME FOR DETECTING ANALYTES

This application claims the priority of U.S. provisional application serial no. 60/145,856 filed on July 28, 1999, the disclosure of which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to the field of optical chemical detection of analytes. More particularly, the present invention provides a device wherein the electromagnetic radiation generator provides a substrate for chemical sensors, and wherein the spectroscopic properties of the chemical sensor are modified upon contacting an analyte. The present invention also provides a method for the selective and simultaneous detection and quantitation of analytes, and a method of making the device.

2. Description of the Related Art

Chemical sensors are widely used in clinical diagnosis and biomedical research to selectively detect the presence of a particular analyte or ensemble of analytes, or to measure other characteristics of samples, such as pH. These measurements are based on the principle that interaction of a chemical sensor with an analyte within a sample results in modification of spectroscopic properties of the sensor to a degree that depends on the concentration of the analyte. The modification of spectroscopic properties may involve changes in the intensity, wavelength, phase, or polarization of the incident electromagnetic radiation. For example, fluorophores are molecules that absorb light at certain wavelengths and emit light of a different

wavelength (generally longer). In the presence of an analyte, the optical properties of some fluorophores are altered and this forms the basis for optical detection and quantitation of analytes using fluorophores.

Many devices disclosed previously use one or more fiber optic strands having a chemical sensor or sensor element at its tip. Some devices use an array of optical fibers to detect the presence of a substance in a sample. One such array disclosed in U.S. Patent No. 5,320,814 has two discrete optic array ends, each of which is formed of multiple end faces of the optical fibers. On one of the optic array ends is a light energy absorbing dye disposed as an uninterrupted deposit in aligned organization upon the end faces.

Another optic sensor is disclosed in U.S. Patent No. 5,512,490 (`490). The device comprises a supporting member and an array formed of heterogeneous semi-selective thin films which function as sensing receptor units and are able to detect a variety of different analytes and ligands using spectral recognition patterns. The supporting member may be a "supporting substrate" which is a translucent or transparent article such that light energy may pass through without being substantially altered or hindered. As shown in Figure 2 of the `490 patent, the receptor units are formed on the supporting substrate and white light from a separate excitation source, such as an arc lamp, and a dichroic mirror are used to illuminate each receptor unit. Alternatively, the supporting member may be a collection of optical fibers, each of which is coated with a polymer/dye combination on a distal tip. As shown in Figure 23 of the `490 patent, light from a separate excitation source in combination with a dichroic mirror is introduced into the optical fibers to illuminate the polymer/dye combination.

These and other existing devices are expensive, and bulky. Furthermore, these devices require a large

amount of energy to operate, in part because the excitation light source is separate from the chemical sensor/sensor element.

SUMMARY OF THE INVENTION

The present invention provides an electromagnetic radiation (ER)-based sensor device that is simple, easy to make and is compact compared to existing devices. While any ER generator may be used for the present invention, in a preferred embodiment, the ER generator is a modified LED (light emitting diode) having micro-wells on its surface. The individual micro-wells are filled with one or more chemical sensing materials so as to form a sensor array.

Thus, an object of the present invention is to provide an ER-based sensing device that is compact and energy efficient for detecting the presence of one or more analytes in samples.

Another object of the present invention is to provide an ER-based sensing device for the simultaneous detection and quantitation of one or more analytes in a sample.

Another object of the present invention is to provide a method for detecting the presence of one or more analytes in a sample.

Another object of the present invention is to provide a method for detecting and simultaneously quantitating one or more analytes in a sample

Yet another object of the present invention is to provide a method of making an ER sensor and sensor array for the detection and quantitation of one or more analytes in a sample.

A detecting device according to the present invention comprises an ER generating substrate having a chemical sensor for interacting selectively with a particular analyte in a sample. In the absence of the analyte, the chemical sensor displays certain baseline

spectroscopic properties characteristic of the sensor. However, when the analyte is present in the sample, the spectroscopic properties of the chemical sensor are modified. Detection and quantitation of the analyte are based on a comparison of the modified properties and the baseline properties and the use of standard calibration methods that are well known to those skilled in the art of analytical chemistry.

The present invention also includes a method of making the detecting device. In the method of making the device according to the present invention, micro-wells are formed on an ER substrate and a chemical sensor and/or sensor element is placed therein in a suitable holding material.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1 and 2 are schematic representations of a side view and a top view respectively of the detecting device according to the present invention.

Figures 3 and 4 are schematic representations of side views of two embodiments of the detecting device according to the present invention.

Figure 5 is a schematic representation of the detecting device according to the present invention in combination with a receiving and interpreting system.

Figures 6 and 7 are schematic representations of a top view and a side view respectively of a detecting device according to the present invention in combination with a flow cell.

Figures 8a and 8b show steps of two methods for making the detecting device according to the present invention.

Figure 9 shows steps of a method of detecting the presence of an analyte in a sample using an electromagnetic radiation generating substrate according to the present invention.

Figure 10 shows the luminescence intensity, as a function of time, from a single micro-well located on the face of an LED according to the present invention having the chemical sensor tris(4,7-diphenyl-1,10-phenanthroline) ruthenium (II) sequestered within a holding material.

Figure 11 shows the fluorescence intensity of electromagnetic radiation emitted by the sensor of Figure 10 in response to gaseous O_2 (a quencher), N_2 (a non-quencher), and air (partial quencher).

Figures 12a and 12b show plots of the activity of the chemical sensor, Glucose oxidase (Gox) for the detection of glucose. Activity is shown in the form of K_m(M) in Figure 12a and as $k_{cat}(s^{-1})$ in Figure 12b for GOx's behavior in the presence of its substrate, glucose, when it is dissolved in buffer (A)-(from *J. Biol. Chem.* 1967, *242*, 994 and *Biochemistry* 1971, *10*, 4624) or sequestered within a tetramethylorthosilane (TMOS) derived xerogel glass (B) - (*Chem. Mater.* 1992, *4*, 1615) or when GOx is held within a micro-well that uses a TMOS-derived xerogel glass as the holding agent as a function of storage time within the xerogel-filled micro-well(C) - 1 month of storage at ambient conditions; (D) - 8 months of storage at ambient conditions).

Figure 13 shows a plot of the affinity constant of the chemical sensor, anti-dansyl antibodies, for its hapten, dansyl, when it is dissolved in buffer - (from *J. Mol. Biol.* 1970, *51*, 573 (A) and *Biochemistry* 1981, *20*, 4624 (B)); when the antibodies are dissolved in buffer (C); or when the anti-dansyl antibodies sequestered within a micro-well that uses a hybrid TMOS-based xerogel glass as the holding agent as a function of storage time within the xerogel-filled micro-well((D) - 1 month of storage at ambient conditions;(E) - 2 months of storage at ambient conditions;(F) - 3 months of storage at

ambient conditions; (G) - 8 months of storage at ambient conditions).

Figure 14 shows the relative fluorescence intensity of electromagnetic radiation emitted by a sensor according to the present invention having fluorescein labeled calmodulin to selectively detect the presence of Ca^{2+} in solution.

Figures 15, A and B show a fluorescence image of a micro-well array on the face of an LED having a fluorescein-labeled monoclonal antibody that is selective to benzo[a]pyrene in the absence (B) or presence (A) of 150 pM B[a]P to detect the presence of benzo[a]pyrene in solution. The signal to noise ration at 15nM was 78.

Figure 16 is a plot of the intensity of fluorescence from a single micro-well located on the face of a LED having GOx as the chemical sensor, in the presence and absence of glucose.

Figures 17-19 shows plots of fluorescence versus concentration of three different analytes, glucose, tyrosinase, and cholesterol, for three discrete micro-wells on the face of a single LED according to the present invention containing the chemical sensors GOx (Fig 17), L-amino acid oxidase (L-AAO) (Fig 18), and cholesterol oxidase (ChOX) (Fig 19).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The term "chemical sensor" or "chemical sensors" as used herein means a molecule or molecules that detect(s) the presence of an analyte. The chemical sensor comprises a sensor element whose optical properties are modified in the presence of an analyte. The properties of the sensor element may be directly modified upon its interaction with the analyte. Alternatively, the sensor element may be attached to a molecule having a specific affinity for the analyte, in which case, the optical properties of the sensor element are modified upon the interaction of the affinity molecule with the analyte.

Thus, by the term "spectroscopic properties of the chemical sensor" or "chemical sensor's spectroscopic properties" is meant the spectroscopic properties of the sensor element and vice versa. These properties may be optical in nature when the emitted electromagnetic radiation is within the visible spectrum i.e., between about 400nm to about 800nm. As an example if the chemical sensor is a fluorescein tagged antibody, the sensor element is fluorescein and the affinity molecule is the antibody. In another example, where the chemical sensor is a luminescent ruthenium dye ($[Ru(dpp)_3]^{2+}$), the sensor element and the chemical sensor are the same.

The present invention provides a compact and energy efficient ER sensor array. The device can also be used for the simultaneous detection and quantification of one or more analytes in a sample. The device comprises an ER generator having one or more micro-wells or zones for placement of chemical sensors. The ER generated by the generator is such that at least some of it can be absorbed by a phosphore, fluorophore, and/or chromophore of the chemical sensor. To be absorbed by the luminophore (fluorophore or phosphore) or chromophore requires that the wavelength range output from the generator overlap at least partially with one or more allowed electronic transitions within the chemical sensor or sensor element. Typically the electromagnetic radiation capable of exciting and/or populating upper electronic transitions in a substance fall within a wavelength region of 200nm to 900nm and thus includes, ultraviolet, visible and infrared portions of the electromagnetic spectrum.

A unique feature of the device is the placement of chemical sensor directly on the ER generator in an array or pattern. This eliminates the need for optical fibers to carry the signal from the ER generator to the chemical sensor, it improves the efficiency of delivery of electromagnetic radiation from the generator to the

chemical sensor, it minimizes alignment problems, and it lowers the necessary fluence from the generator which leads to the use of smaller and lower power (e.g., battery operated) generators.

The ER generator can be any device that generates electromagnetic radiation of a wavelength that will cause electronic transitions in a chemical sensor such as light emitting diodes and diode lasers. In a preferred embodiment, the electromagnetic radiation generator is light emitting diode (LED).

The chemical sensor can be placed directly on the ER generator or it can be placed within micro-wells created on the surface of the generator. The number, size, and shape of the micro-wells on an ER generator can vary. While any ratio of micro-wells to non micro-well area is suitable, a ratio of 1:4 generally ensures that individual wells are reasonably well separated from one another. For example, on an LED of 5 mm diameter, having 10 μm diameter wells, with a 1:4 ratio of micro-wells to non-micro-well area, it is estimated that 62,000 micro-wells can be formed. Each micro-well may contain a different chemical sensor so that the same LED may be used for the simultaneous detection and quantitation of multiple analytes.

To fill the micro-wells with the chemical sensor, a filling or holding material is used. Any material known to those skilled in the art for holding, immobilizing, entrapping, and/or sequestering chemical sensors, can be used. These materials include, but are not limited to, sol-gel derived materials, acrylamide gels, small particle beads and surface-immobilized species. One commonly used holding material is a sol-gel-derived glass. A sol-gel-derived glass is a porous glass formed by the condensation and polycondensation of one or more metal or semi-metal alkoxide mixtures. Sol-gel-derived glasses provide a convenient means to sequester sensors, and/or sensing agents, because they prevent leaching from

the holding material, and the glasses themselves are porous, thereby allowing analytes to penetrate into the glass, and react with the chemical sensors. Glasses with surface areas of up to several hundred square meters per gram and narrow pore diameters (0.5 to 500nm) are readily prepared using sol-gel methods well known to those skilled in the art of sol-gel processing chemistry. A detailed discussion of sol-gel chemistry can be found in Reisfeld et al., 1992, *Chemistry, Spectroscopy and Application of Sol-Gel glasses,* Springer-Verlag, Berlin; Brinker et al., 1989, *Sol-Gel Science,* Academic Press, New York; Dave et al., 1994, *Anal. Chem.* 66:1120A, 1121A.

It is preferred that the mean pore diameter be less than the mean wavelength of electromagnetic radiation from the generator, but deviation leads only to a predictable decrease in performance. The sol-gel-derived glass useful in the present invention is preferably transparent or translucent for wavelengths of from about 300 nm to about 900 nm. Translucent materials preferably have a transmittance of 50% or greater.

Chemical sensors may simply be added to the sol-gel-derived glass holding material once the sol-gel-derived glass is placed or located or formed in the micro-wells, or they may be doped into the sol-gel processing solution (precursor to the glass and/or xerogel) before it is filled into the micro-wells. A property that makes sol-gel-derived glass useful for the present invention is that molecules sequestered within the glass may interact with diffusible analytes or components in an adjacent liquid or gas phase within the glass pore space. In addition to sol-gel-derived glass, other organic or inorganic polymers and mixtures thereof that can be easily filled into the micro-wells and remain within the wells, can also be used as holding materials.

Chemical sensors or sensor elements that are useful for the present invention include materials whose

spectroscopic properties are modified due to interaction with specific analytes. The modification of spectroscopic properties may include a change in wavelength, intensity, phase, and/or polarization of the incident electromagnetic radiation.

Materials that cause a change in the wavelength of incident (exciting) ER are referred to as fluorophores or phosphores and typically absorb ER of a particular wavelength and emit ER of a different wavelength. The absorption and emission spectra are characteristic for each fluorophore or phosphore. Materials that absorb electromagnetic radiation and do not fluoresce, generally convert any excess energy produced as a result of photoexcitation into heat energy or kinetic energy and are referred to as chromophores. Many dyes are known in the art that absorb electromagnetic radiation of a specific wavelength.

The detection of the transmitted or emitted electromagnetic radiation from the chemical sensor may be carried out by collecting the electromagnetic radiation from each individual micro-well with an objective, passing it through a filter system and ultimately communicated to a solid state array detector, such as a charge coupled device (CCD).

The following examples are presented for illustrative purposes and are not to be construed as limiting.

Figures 1 and 2 illustrate an LED having micro-wells thereon. LEDs are typically covered with a protective coating. Thus, the detecting device 10 according to the present invention, includes an ER generator 13 in contact with the protective layer 16. The protective layer 16 is a substance which is transparent or translucent to electromagnetic radiation generated by the substrate 13. Preferably, the transmittance is 50% or greater. The protective layer 16 has one or more micro-wells 19 formed in a distal end 22 of the protective layer 16. The

micro-wells 19 preferably extend into the protective layer 16, but not through the protective layer 16 to the point that they contact the actual LED p-n junction. The LED generates electromagnetic radiation when an electric potential is applied via the conductors 25. Any commercially available LED can be used for this invention. The primary consideration is that the electromagnetic radiation emitted by the LED be at least partially absorbed by the chromophore(s), fluorophore(s), and/or phosphore(s) that comprise the sensor(s).

Within each discrete micro-well 19 is a combination 28 of a holding material and a chemical sensor, which is capable of selectively interacting with the analyte to be detected and quantified. When the LED generates electromagnetic radiation, the chemical sensor displays a characteristic spectroscopic property. For example, the chemical sensor may emit light of one wavelength in the absence of the analyte. When the chemical sensor interacts with the analyte, its optical properties are modified, and it may emit light of a second wavelength. In some cases, an analyte may interact with a chemical sensor to change its intensity or polarization of fluorescence. The change in the intensity or polarization may involve an increase or decrease. It should be noted that the sensor element of the chemical sensor may itself interact with the analyte or alternatively, the sensor element may be attached to another molecule or fragment of a molecule that interacts selectively with the analyte.

The chemical sensor is held within a micro-well 19 by a transparent or translucent holding material, preferably with a transmittance of 50% or greater, which may be any of the organic or inorganic polymers, or mixtures thereof, which are well known in the art for sequestering chemical sensors. Preferably, the holding material is a sol-gel-derived glass which forms a porous

xerogel or aerogel upon setting in the well 19. One such holding material suitable for use in the present invention is a sol-gel-derived glass comprised of TMOS.

Figures 3 and 4 depict another embodiment of the present invention in which no well is used. In the embodiment shown in Figure 3, the chemical sensor is placed directly on a substantially planar surface 34 of the protective layer 16. The chemical sensor may be placed on the planar surface 34 by using a micropipette or microinjector to deliver the sensor and its holding material on the planar surface 34. Alternatively, the chemical sensor may be placed directly on the substrate 13, as shown in Figure 4, in the absence of a protective layer. The chemical sensor may be fixedly attached to the substantially planar surface 34 of the protective layer 16 by mixing it with the sol-gel-processing solution described above, and then placing the combination 28 on the planar surface 34, where the combination is allowed to set.

The chemical sensors of the present invention comprise a sensor element, whose optical properties are modified in the presence of an analyte. Sensor elements that can be used for the present invention include electromagnetic radiation absorbing and electromagnetic radiation emitting inorganic or organic dyes (either natural, synthetic, or combinations thereof). Such dyes include phosphores, fluorophores, and chromophores. Many luminescent and chromogenic molecules are well known to those skilled in the art. Examples of such materials are disclosed in U.S. Patent no. 5,250,264. Other sources of useful chemical sensors or sensor elements include the Handbook of Fluorescent Probes and Research Chemicals, 6th ed., authored by Richard P. Haugland and published by Molecular Probes, Inc. of Eugene, Oregon. As discussed above, some of the chemical sensors absorb light emitted from the LED in the presence of an analyte to a degree that depends on the analyte concentration, while others

luminescence to a degree that depends on the analyte concentration in the presence of the analyte to be detected and/or quantified. Also as mentioned above, the sensor element may directly detect the analyte or may indirectly detect the analyte through an affinity molecule. Such affinity molecules will have substantial affinity for the analyte and include inorganic or organic ligands; inorganic or organic chelators; proteins, including antibodies, enzymes and binding proteins; and nucleic acids. These molecules may be natural or synthetic.

The types of analytes that may be detected include both liquid and gaseous materials. These include CO_2, O_2, pesticides, drugs, herbicides, anions, cations, antigens, oligonucleotides, and haptens. Further, the present invention can indicate the pH of a sample. In addition, chemical sensors are available and can be used in the present invention to detect the presence of organic molecules such as polycyclic aromatic hydrocarbons, glucose, cholesterol, amino acids, peptides, DNA and RNA. There are many more substances which can be detected, and the foregoing list is not to be considered exhaustive, but instead is merely representative.

The electromagnetic radiation emitted by the chemical sensor may be detected by any suitable method known in the art. A general configuration is illustrated in Figure 5, which shows a detecting device 10 according to the present invention in combination with a receiving and interpreting system 37. The receiving and interpreting system 37 has a receiver to receive electromagnetic radiation transmitted or emitted by the chemical sensor and an interpreter to interpret the received radiation. The receiver shown in Figure 5 includes a lens or series of lenses 40, a filter 43 and a receiving surface 46. A suitable receiver is a microscope objective. The receiver may have a camera for recording images. The interpreter includes a controller

49 and a computer 52 having software running thereon. The receiving surface 46 is connected to the controller 49 via first communication line 55. The controller 49 is connected to the computer via second line 58.

An example of a device having a series of lenses 40, is a standard inverted fluorescence microscope. An example of a microscope suitable for use in the present invention is, model number BX-FLA available from Olympus America, Inc. of Melville, New York.

The receiving surface 46 may be a charge coupled device, which may be part of a CCD camera. An example of a CCD camera which can be used in the present invention is model number TE/CCD-1317K manufactured by Princeton Instruments, Inc. of Trenton, New Jersey. An example of a controller 49 which is suitable for use in the present invention is model number ST-138 manufactured by Princeton Instruments.

A filter 43 may be placed between the substrate 13 and the receiving surface 46. The filter 43 selectively passes desired wavelengths of the electromagnetic radiation moving from the detecting device 10 toward the receiving surface 46 and blocks undesired wavelengths. An example of a filter 43 which can be used to practice the present invention is model number XF 3000-38 manufactured by Omega Optical of Brattleboro, Vermont. This particular filter passes electromagnetic radiation above approximately 515 nm and strongly attenuates electromagnetic radiation below approximately 515 nm. Other filters or filter combinations are possible depending on the generator wavelength and the particulars associated with a given sensor.

Figures 6 and 7 depict an embodiment of the present invention in which a detecting device 10 according to the present invention is positioned within a flow cell 67. The flow cell 67 permits continuous monitoring of a stream of sample or discretely injected plugs from multiple samples. The flow cell 67 has an inlet 70, a

channel 73 and an outlet 76. A sample to be analyzed is provided at the inlet 70, flows through the channel 73 in the direction indicated by arrows 79, and finally leaves the channel 73 via the outlet 76. As the sample flows over the detecting device 10, the sample contacts and interacts with the chemical sensor(s).

It will be recognized by those skilled in the art that a flow cell 67 need not be provided to practice the present invention. The chemical sensor(s) is merely contacted with a sample to be analyzed, and then placed in the proper position to permit the receiving and interpreting system 37 to receive radiation from the chemical sensors. Consequently, in lieu of using the flow cell 67, the detecting device 10 may be dipped in a sample and then properly positioned relative to the receiving and interpreting system 37.

The device of the present invention can be made by preparing micro-wells on the surface of an ER generator. Steps for preparing a device according to the present invention are illustrated in Figure 8a. As discussed above, a suitable ER generator is an LED (Step 100). It is preferable to have a planar surface on the LED for making the micro-wells (Step 103). Thus, if the LED has a non-planar tip, a portion of it may be removed to provide a substantially planar exposed surface. The micro-wells can be formed either on the protective layer (step 106) that is generally present on the LED or they can be formed on the LED after removal of some or all of the protective layer. The wells are preferably formed by any micromachining methodology. Examples of micromachining methods include mechanical drilling with small diameter drill bits, chemical etching/lithography, and/or laser-based drilling with a continuous wave or pulsed laser by free hand or with a predetermined pattern (Step 106). Alternatively, the protective layer may be molded to have the micro-wells as an intrinsic part of the LED, thereby alleviating the need to remove portions

of the protective layer and micromachining steps to form a well. A suitable well depth is from 0.1 mm to 1 mm, but other depths are suitable for certain applications where faster response time and/or greater overall signal-to-noise is required. A micro-well can be formed by mechanically drilling into the LED to a defined depth. The depth is controlled by mechanically translating the drill and/or LED on a lathe and or drill press. The LED and drill can then be moved relative to one another, the next well is drilled, and so on until an array of micro-wells on the LED face is formed. A similar strategy can be used for laser-based drilling where the laser beam and/or the LED can be translated with respect to one another to effect a pattern. Here the laser beam fluence, laser illumination time on the LED face, and/or laser beam focal point waist can all be used to precisely control the micro-well depth, well position, and well diameter. One can also use the laser-based method in concert with a template to micromachine a pattern or array of micro-wells on the face of an LED or other generator. Next, a sol-gel-processed solution is added into the micro-wells using a micro-pipette and/or a microinjector with a micromanipulator. A defined volume is added into each micro-well. The contents of each micro-well are allowed to age/cure for a defined time that depends precisely on the well depth, its diameter, and the exact composition of the holding agent phase. The chemical sensor is prepared (Step 109) and may be added to the micro-wells using a micropipette or a microinjector after the wells are filled with the sol-gel-derived glass or it can be mixed directly with the sol-gel-processing solution before filling (Step 112).

In one embodiment, the device may be prepared without creating micro-wells. Thus, the steps illustrating this embodiment are presented in Figure 8b. A suitable ER generator is provided (with or without the protective layer) (Step 150). It is preferable to have a

planar surface on the LED for deposition of the chemical sensor (Step 153). If the LED has a non-planar tip, a portion of it may be removed to provide a substantially planar exposed surface. The chemical sensor is prepared (Step 156) mixed with the sol-gel-processing solution for deposition (Step 159).

The detecting device 10 described herein and the receiving and interpreting system 37, as illustrated in Figure 5 can be used to practice a method of the present invention. The method comprises the steps of obtaining a baseline reference of the desired spectroscopic property of the chemical sensors. For example, the fluorescence intensity or the fluorescence wavelength from an individual micro-well or array of micro-wells on the face of an LED having a chemical sensor or array of sensors may be recorded. Then the detecting device 10 is contacted with a sample containing one or more target analytes. The spectroscopic properties of the contacted chemical sensor are recorded again and compared to the baseline reference. Any detectable deviation of the spectroscopic properties from the baseline indicates the presence of the analyte. The concentration of the analyte is obtained by comparing the deviation of the spectroscopic properties from the baseline and the sample to the deviation observed from a calibrated set of known standards. Those skilled in the art will recognize that the concentration or quantity of analyte in the sample may also be obtained without determining the deviation of the spectroscopic properties from the baseline by simply comparing the spectroscopic properties of the chemical sensor in the presence of the analyte with a calibrated set of standards.

An illustration of the steps involved in the detection of analytes is presented in Figure 9. The ER generating substrate is provided (Step 200). For operation, a signal is generated from the ER generating substrate which has micro-wells containing an ensemble of

discrete chemical sensors (Step 203). Emitted radiation from the chemical sensors is received (Step 206), focused (Step 209) and received at the receiving surface (Step 212). The receiving surface then generates a first signal corresponding to the received radiation (Step 215). The first signal is transmitted on the first communication line 55 to the controller 49 (Step 218). The controller 49 in turn generates a second signal corresponding to the first signal (Step 221), and provides the second signal on the second communication line 58 (Step 224). The second signal is formed by the controller 49 to conform to a transmission format understandable by the computer 52 (Step 227). Then the computer 52 receives the second signal via the second communication line 58 and processes the second signal using software running on the computer 52 to provide (Step 230) a processed second signal in the form of useful information about the radiation received by the receiving surface 46 to the analyst 64.

The following specific embodiments describe the use of the present invention in the detection and quantitation of analytes.

Example 1

This embodiment illustrates the preparation of one sol-gel composition suitable for the present invention. It should be recognized that this is a specific description of the preparation of a particular sol-gel-derived glass material. Other sol-gel-derived materials can be prepared using obvious variants of this method based on the information provide herein and by using protocols that are known in the art of sol-gel chemistry. The Ru(dpp)32+-Doped Sol-gel-derived thin films were prepared as follows. An acid-catalyzed sol-gel-processed stock solution was prepared by mixing TMOS (15 mmole), deionized water (30 mmole), EtOH (30 mmole), and HCl (15 x 10-4 mmole). This solution was stirred

under ambient conditions for 4 h. The mixture was then transferred into a clean glass vial. Fifty microliters of [Ru(dpp)3]2+ dissolved in EtOH. The ethanolic [Ru(dpp)3]2+ solutions contained approximately 100 micromoles of Ru(dpp)32+. The solutions were allowed to stir for 1 h. The solution were transferred into microwells and allowed to age under ambient conditions for 2 days.

Example 2

This embodiment demonstrates the stability of the chemical sensor in a holding material according to the present invention. A sol-gel-derived xerogel containing tris(4,7-diphenyl-1,10-phenanthroline) ruthenium (II) ($[Ru(dpp)_3]^{2+}$), an organometallic luminescent molecule, was prepared as described above. The dye concentration was ~25 uM. Microwells on an LED were filled by hand with a micropipette. A typical volume added into a micro-well was ~1-2 uL. Incident light of ~470 nm was produced by the LED, this light excited the $[Ru(dpp)_3]^{2+}$-doped xerogel that filled the micro-wells and the $[Ru(dpp)_3]^{2+}$ luminesced. The luminescence output from a single micro-well was monitored continuously while the sensor was operating in air. As shown in Figure 10, the intensity of luminescence was constant over a 3000 second period under constant operation and there was no evidence of any dye photobleaching. Thus, this data demonstrates that using the method of the present invention, the chemical sensor is sufficiently stable to be used for detection and quantitation.

Example 3

This embodiment demonstrates the reliability of the device and method of the present invention. In one illustration of this embodiment, the selectivity of a response using a chemical sensor is demonstrated. The LED from Example 1 was contacted with analytes whose

effects on this chemical sensor are known. Oxygen is known to quench the luminescence of this chemical sensor while nitrogen is known not to have any effect. Figure 11 shows the response profile upon repeated challenge of a $[Ru(dpp)_3]^{2+}$-doped xerogel-filled micro-well on an LED face when the sample stream is switched from O_2 (low fluorescence) to N_2 (higher fluorescence) to air (intermediate fluorescence). Thus, this embodiment illustrates reversibility, reproducibility, selectivity, and response time of the device and method of the present invention.

Example 4

This embodiment demonstrates the sensor stability, for storage purposes, of a micro-well that is filled with a sol-gel-derived glass containing a chemical sensor. Figure 12 shows a comparison of the performance of the enzyme glucose oxidase (GOx) dissolved in aqueous solution (A) or TMOS (B), or sequestered within a sol-gel-derived glass that has been stored for 1 month (C) and eight months (D). As shown in Figure 12, GOx, sequestered within a sol-gel-derived glass within a micro-well, is reasonably stable for at least 8 months.

In another illustration of this embodiment, the activity of an antibody was tested in the sol-gel-derived glass that was within a micro-well for various times of storage. Figure 13 shows the affinity constant for a hapten-antibody complex, dansyl/anti-dansyl, sequestered in the sol-gel-derived glass according to the present invention as a function of storage time. Measurements are shown for the same micro-well sensor after storage for 1, 2, 3, and 8 months. Thus, this experiment illustrates that the antibody affinity is not significantly affected when it is sequestered within the xerogel glass for prolonged periods of time and array sensors based on antibodies are possible.

Example 5

This embodiment illustrates that the device and method of the present invention can be used to detect and quantitate analytes. In the present experiment, a sensor element was attached to a molecule having a specific affinity for an analyte. For this experiment, calmodulin was site selectively labeled with a fluorescent molecule, fluorescein. The precise position of the fluorophore and the synthetic strategy used to prepare the fluorescein-labeled calmodulin (CaM-F) have been reported in A.N. Watkins and F.V. Bright, "Effects of Fluorescent Reporter Group Structure on the Dynamics Surrounding Cysteine-26 in Spinach Calmodulin: A Model Biorecognition Element," *Appl. Spectrosc.* 1998 *52*, 1447, which disclosure is incorporated herein by reference. The microwells on the face of an LED were filled with a TMOS-based sol-gel-processing solution that contained ~2 μM CaM-F and the fluorescence intensity from the micro-well was measured in the presence of various concentrations of free calcium ion. The relative fluorescence intensity as a function of time for CaM-F within a xerogel, within a micro-well as it is challenged with increasing concentrations of calcium ion is shown in Figure 14. "Apo" refers to Apo-CaM which represents the state when no calcium ion is present to bind to CaM. At the three time points, the LED sensor was exposed to free calcium ion concentrations of 7 nM, 16 nM and 27 nM. As seen in Figure 14, a rapid and measurable response is observed at each concentration illustrating that the device of the present invention can be used with protein-based recognition chemistries to selectively detect analytes.

In another illustration of this embodiment, an analyte was detected by using a sensor element attached to a monoclonal antibody specific for the analyte. A monoclonal antibody to benzo[a]pyrene (B[a]P) was labeled

with fluorescein by methods well known in the art. Figure 15 (panels A and B) shows an image of a micro-well array in the presence and absence of B[a]P dissolved in solution. In this particular reaction, the fluorescein residue fluorescence from its site(s) on the monoclonal anti-B[a]P antibody was enhanced by the binding of B[a]P to the antibody. Thus, the upper panel (A) in Figure 15 represents fluorescence corresponding to a concentration of 150 pM B[a]P while the lower panel (B) represents fluorescence in the absence of B[a]P. The signal-to-noise for a 150 pM concentration of B[a]P was 78. Thus, this experiment illustrates that the device and method of the present invention can be used with antibody-based recognition chemistries, can operate in an array format, and offers detection limits in the low picomolar range.

In another illustration of this embodiment, the presence of glucose in a sample was detected by using glucose oxidase as the chemical sensor. In this particular format, the intrinsic fluorescence from the flavin adenine dinucleotide (FAD) residues that make-up the redox active site within GOx is monitored. Figure 16 presents the fluorescence intensity response of the sensor in the absence of added glucose, after the addition of one 10 mM bolus of glucose and a second 10 mM bolus (20 mM total) of glucose. Figure 16, thus, illustrates that the detecting device 10 can be used with enzyme-based recognition chemistries.

In another experiment, three different micro-wells located on the face of a single LED were filled with sol-gel-processed solutions that were individually doped with the sensors glucose oxidase (Fig 17), L-amino acid oxidase (Fig 18), and cholesterol oxidase (Fig 19) respectively. By exposing these sensors to different concentrations of the appropriate substrates (i.e., glucose, tyrosinase and cholesterol), dose response curves shown in Figure 17-19 were generated. The flavin adenine dinucleotide (FAD) fluorescence was followed in

this particular example so the detection wavelength was identical for each micro-well. Figures 17-19 again illustrates that the device and method of the present invention can be used with enzyme-based recognition chemistries. Figures 17-19 also shows the potential for calibration of the device as well as the use of an array of micro-wells in the simultaneous detection of multiple analytes in the same sample.

It should be apparent to those skilled in the art that the present invention accomplishes the intended objects described above. The present invention provides a detecting device wherein the chemical sensor can be placed in contact with the ER generator, making the device compact. Furthermore, the electromagnetic radiation used in the present invention is not reflected, filtered, or transmitted over a long distance prior to reaching the chemical sensor. In addition, the detecting device according to the present invention can be made relatively inexpensively and readily mass produced.

Although preferred embodiments of the present invention have been described and illustrated herein, the present invention is not limited to such preferred embodiments. Since various changes could be made without departing from the spirit and scope of the invention, it is intended that the foregoing description shall be interpreted as illustrative, and not interpreted in a limiting sense.

What is claimed is:

1. A device for detecting the presence of at least one analyte in a sample, comprising:

an electromagnetic radiation generating substrate;

a protective layer in contact with the electromagnetic radiation generating substrate and having a well formed therein; and

a chemical sensor positioned in the well for reactive contact with the analyte in the sample, and upon receiving radiation from the substrate, the chemical sensor is capable of emitting electromagnetic radiation comprised of a first set of one or more wavelengths when the analyte is present in the sample, and is capable of emitting electromagnetic radiation comprised of a second set of one or more wavelengths, different from the first set, when the analyte is not present in the sample.

2. The device of claim 1, wherein the electromagnetic radiation generating substrate is a light emitting diode.

3. The device of claim 1, further comprising a receiving and interpreting system having electromagnetic radiation receiver to receive electromagnetic radiation emitted by the chemical sensor, and having an interpreter to interpret the received electromagnetic radiation.

4. The device of claim 3, wherein the receiver includes a filter for selectively passing electromagnetic radiation.

5. The device of claim 3, wherein the receiver includes a charge coupled device.

6. The device of claim 5, wherein the receiver includes a lens for focusing the electromagnetic radiation on the charge coupled device.

7. The device of claim 1, wherein the interpreter includes a computer.

8. The device of claim 1, further comprising a holding material for holding the chemical sensor in the well.

9. The device of claim 8, wherein the holding material is a sol-gel.

10. The device of claim 9, wherein the holding material is comprised of tetramethylorthosilane.

11. The device of claim 1, wherein the chemical sensor is comprised of a sensor element and an affinity molecule having a specific affinity for the analyte.

12. The device of claim 11, wherein the affinity molecule is an antibody to the analyte.

13. The device of claim 11, wherein the sensor element is selected from the group consisting of fluorophore, phosphore and chromophore.

14. A device for detecting the presence of an analyte in a sample, comprising:

an electromagnetic radiation generating substrate; and

a chemical sensor positioned on the substrate for reactive contact with the analyte, and upon receiving radiation from the substrate, the chemical sensor is capable of emitting electromagnetic radiation comprised of a first set of one or more wavelengths when the analyte is present in the sample, and is capable of emitting electromagnetic radiation comprised of a second set of one or more wavelengths, different from the first set, when the analyte is not present in the sample.

15. The device of claim 14, wherein the electromagnetic radiation generating substrate is a light emitting diode.

16. The device of claim 14, further comprising a receiving and interpreting system having a receiver to receive the electromagnetic radiation emitted by the chemical sensor and an interpreter to interpret the received electromagnetic radiation.

17. A method of making a detecting device, comprising:
providing an electromagnetic radiation generating substrate in contact with a protective layer;
forming a well in the protective layer;
providing a chemical sensor; and
placing the chemical sensor in the well.

18. The method of claim 17, wherein the step of providing the substrate includes providing a light emitting diode.

19. The method of claim 17, wherein the step of forming a well in the protective layer includes drilling to remove a portion of the protective layer to form the well.

20. The method of claim 17, wherein the step of forming a well in the protective layer includes exposing the protective layer to radiation from a laser to remove a portion of the protective layer to form the well.

21. The method of claim 17, wherein the step of forming a well in the protective layer includes chemically removing a portion of the protective layer to form the well.

22. The method of claim 17, wherein the step of forming a well in the protective layer includes molding the

protective layer to have the well in the protective layer.

23. The method of claim 17, wherein the step of placing the indication material in the well includes filling the well using a pipette.

24. A method of detecting the presence of an analyte in a sample, comprising:

providing an electromagnetic radiation generating substrate having a chemical sensor thereon;

emitting electromagnetic radiation with the substrate;

receiving the emitted electromagnetic radiation with the chemical sensor to cause the chemical sensor to emit radiation;

receiving the radiation emitted by the chemical sensor on a receiving surface;

generating a signal corresponding to the radiation received on the receiving surface;

providing the signal to a computer having software running thereon;

processing the signal using the software running on the computer to generate a processed signal; and

providing the processed signal to an analyst.

25. The method of claim 24 wherein the electromagnetic radiation generating substrate is a light emitting diode.

26. The method of claim 24 further comprising focusing the radiation emitted by the chemical sensor.

27. The method of claim 26 wherein focusing the radiation emitted by the chemical sensor is performed by a lens.

28. The method of claim 24 wherein the receiving surface is a charge coupled device.

29. A method of detecting the presence of an analyte in a sample, comprising:

providing an electromagnetic radiation generating substrate having a chemical sensor thereon;

generating electromagnetic radiation from the substrate such that the chemical sensor emits a first spectroscopic signal;

contacting the chemical sensor with the sample to provide a contacted chemical sensor;

detecting a second spectroscopic signal emitted by the contacted chemical sensor; and

comparing the first spectroscopic signal to the second spectroscopic signal,

wherein a difference between the first spectroscopic signal and the second spectroscopic signal indicates the presence of the analyte in the sample.

30. The method of claim 29 wherein the analyte is glucose.

31. The method of claim 25, wherein the analyte is calcium ions.

32. The method of claim 25, wherein the analyte is cholesterol.

33. A method of detecting and quantitating the presence of an analyte in a sample, comprising:

providing an electromagnetic radiation generating substrate having a chemical sensor thereon;

contacting the chemical sensor with the sample to provide a contacted chemical sensor;

generating electromagnetic radiation from the substrate such that the contacted chemical sensor emits a spectroscopic signal;

detecting the spectroscopic signal emitted by the contacted chemical sensor;

comparing the spectroscopic signal emitted by the contacted chemical sensor to a standard curve to determine the quantity of the analyte in the sample.

34. A method of detecting and quantitating the presence of an analyte in a sample, comprising:

providing an electromagnetic radiation generating substrate having a chemical sensor thereon;

generating electromagnetic radiation from the substrate such that the chemical sensor emits a first spectroscopic signal;

contacting the chemical sensor with the sample to provide a contacted chemical sensor;

detecting a second spectroscopic signal emitted by the contacted chemical sensor;

comparing the first spectroscopic signal to the second spectroscopic signal;

determining a difference between the first spectroscopic signal and the second spectroscopic signal to provide a detectable change; and

comparing the detectable change to a standard curve to determine the quantity of the analyte in the sample.

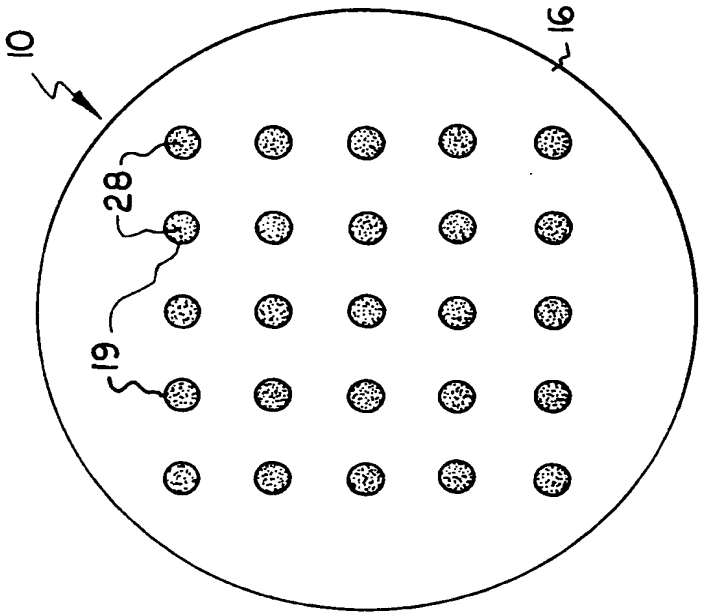


FIG. 2

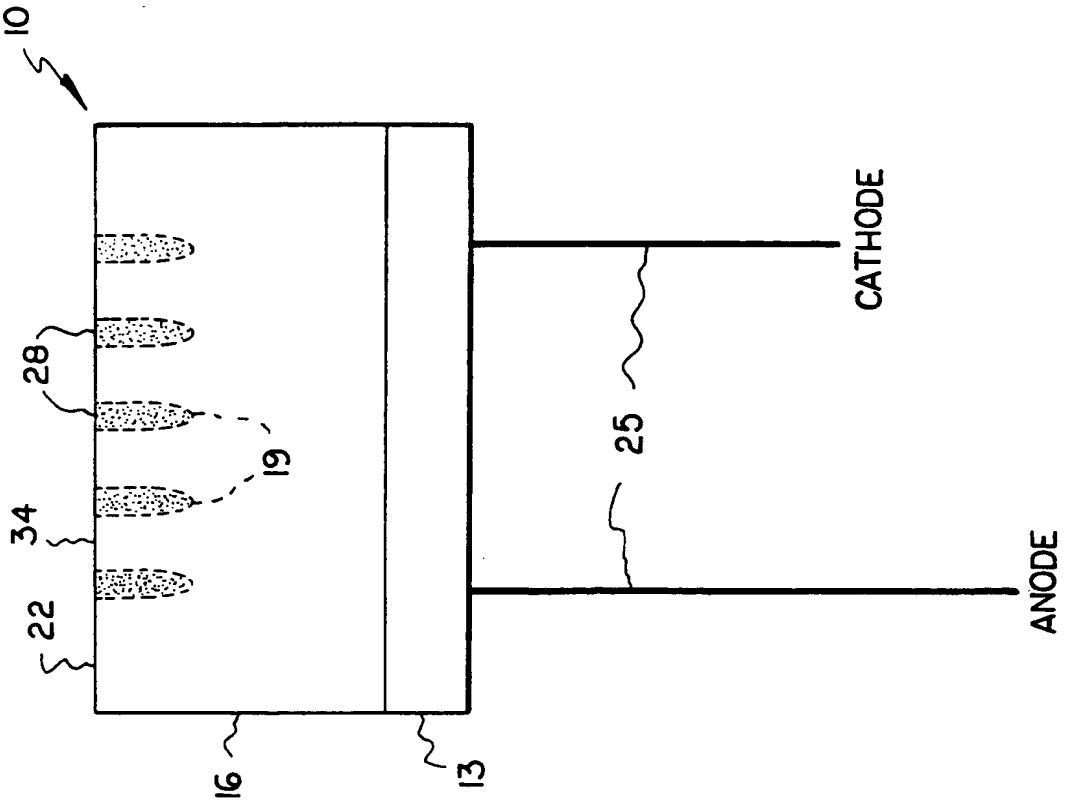


FIG. 1

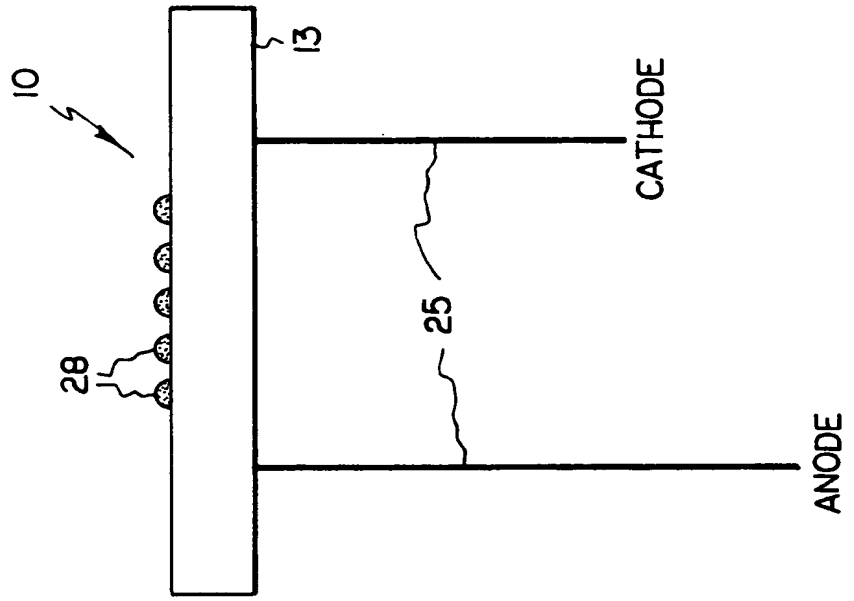


FIG. 4

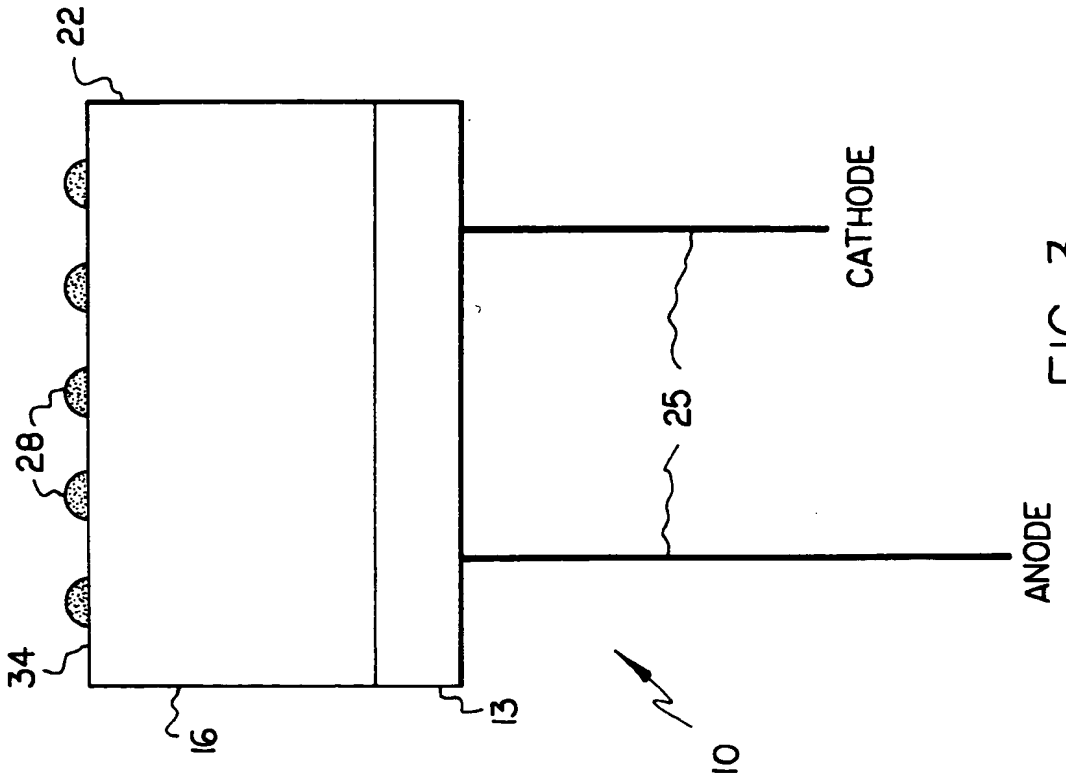


FIG. 3

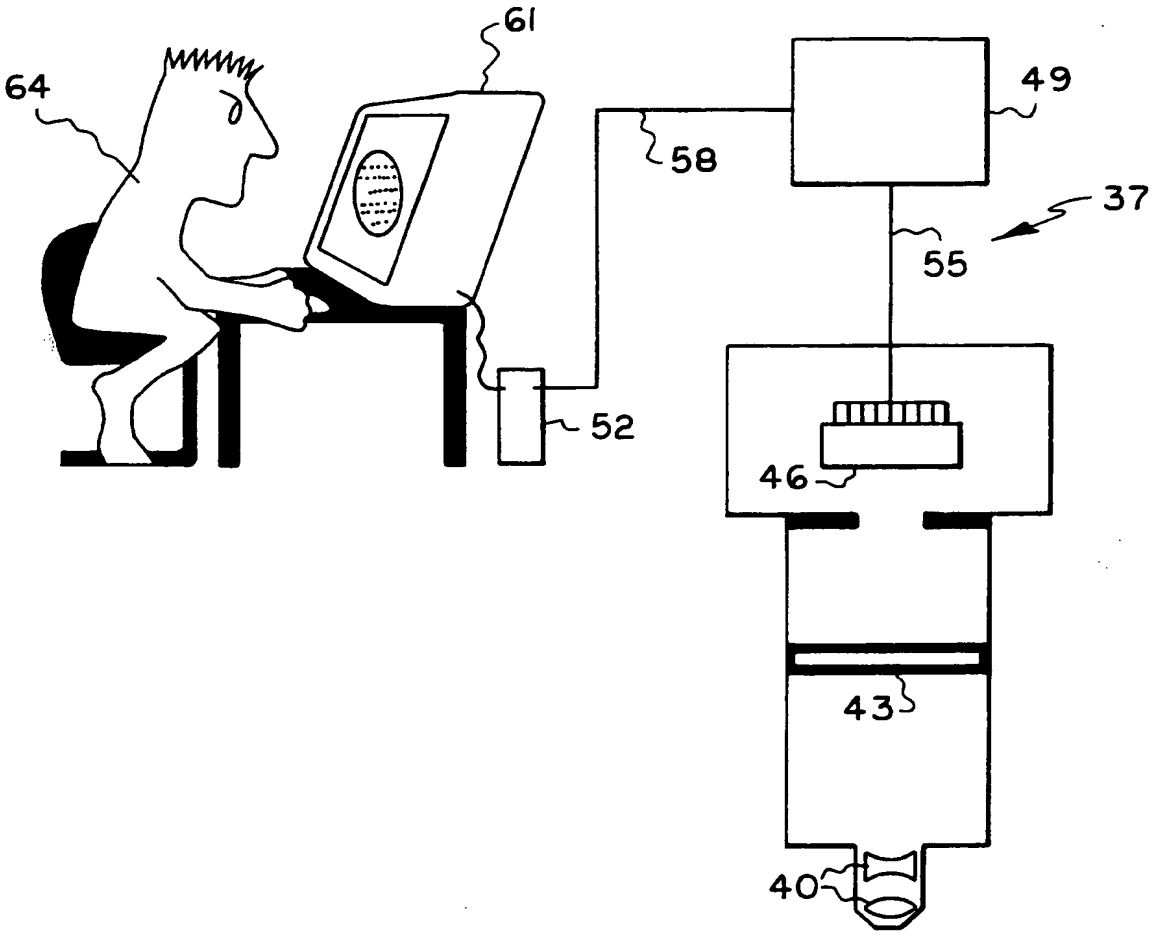
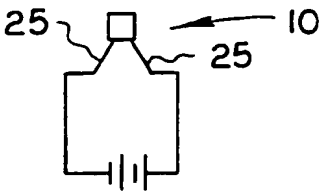


FIG. 5



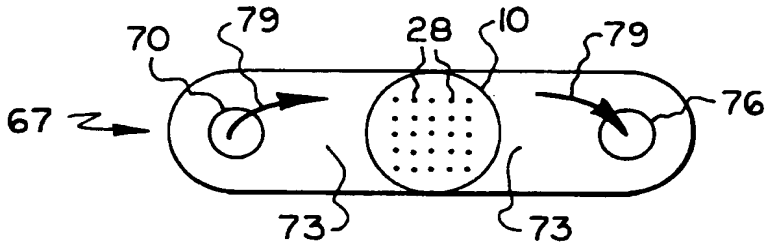


FIG. 6

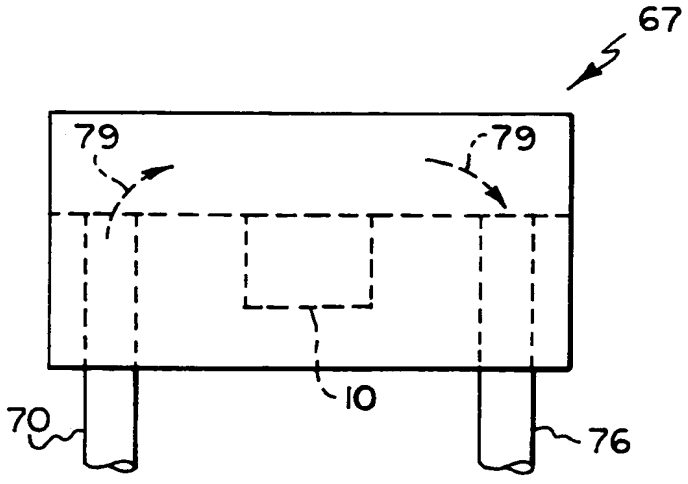


FIG. 7

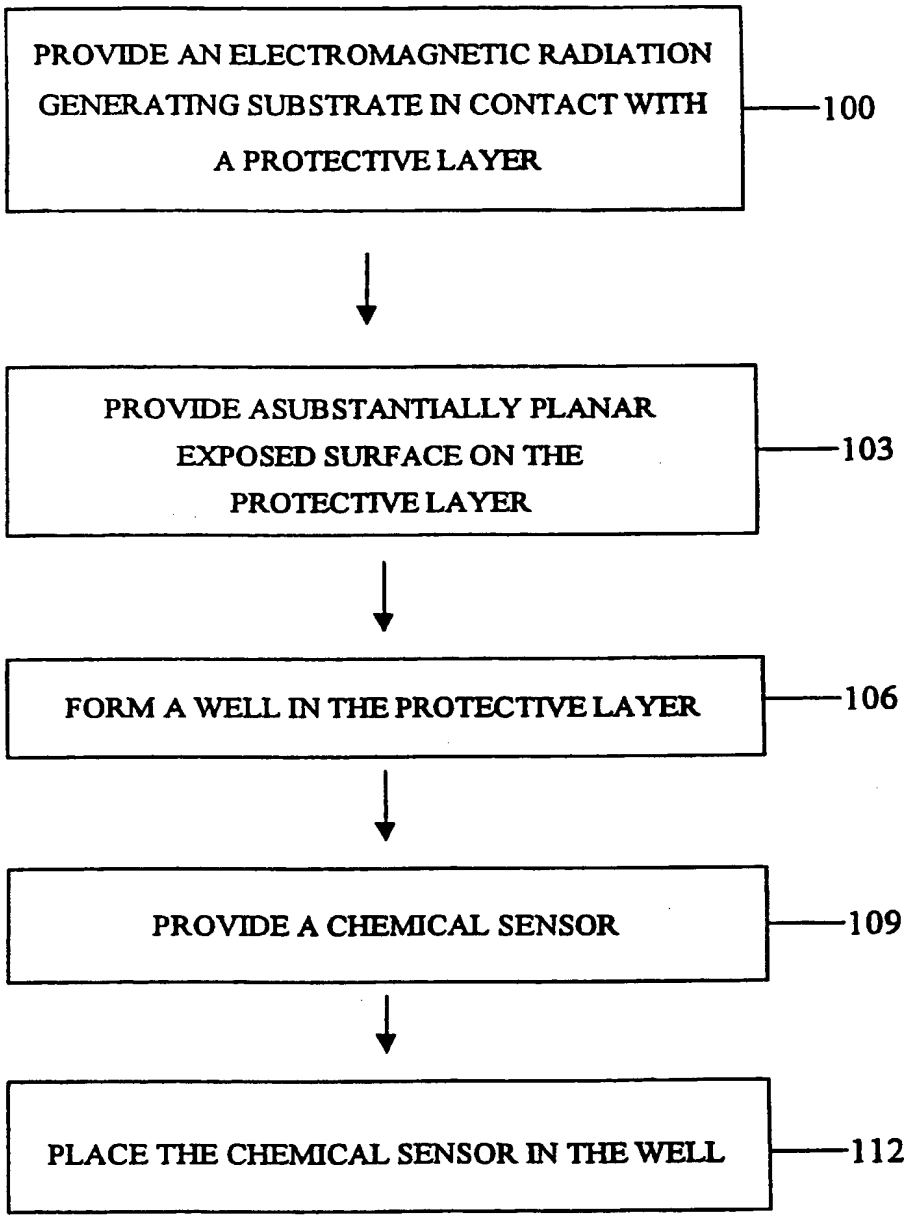

PROVIDE AN ELECTROMAGNETIC RADIATION GENERATING SUBSTRATE IN CONTACT WITH A PROTECTIVE LAYER
100
PROVIDE ASUBSTANTIALLY PLANAR EXPOSED SURFACE ON THE PROTECTIVE LAYER
103
FORM A WELL IN THE PROTECTIVE LAYER
106
PROVIDE A CHEMICAL SENSOR
109
PLACE THE CHEMICAL SENSOR IN THE WELL
112

FIG. 8a

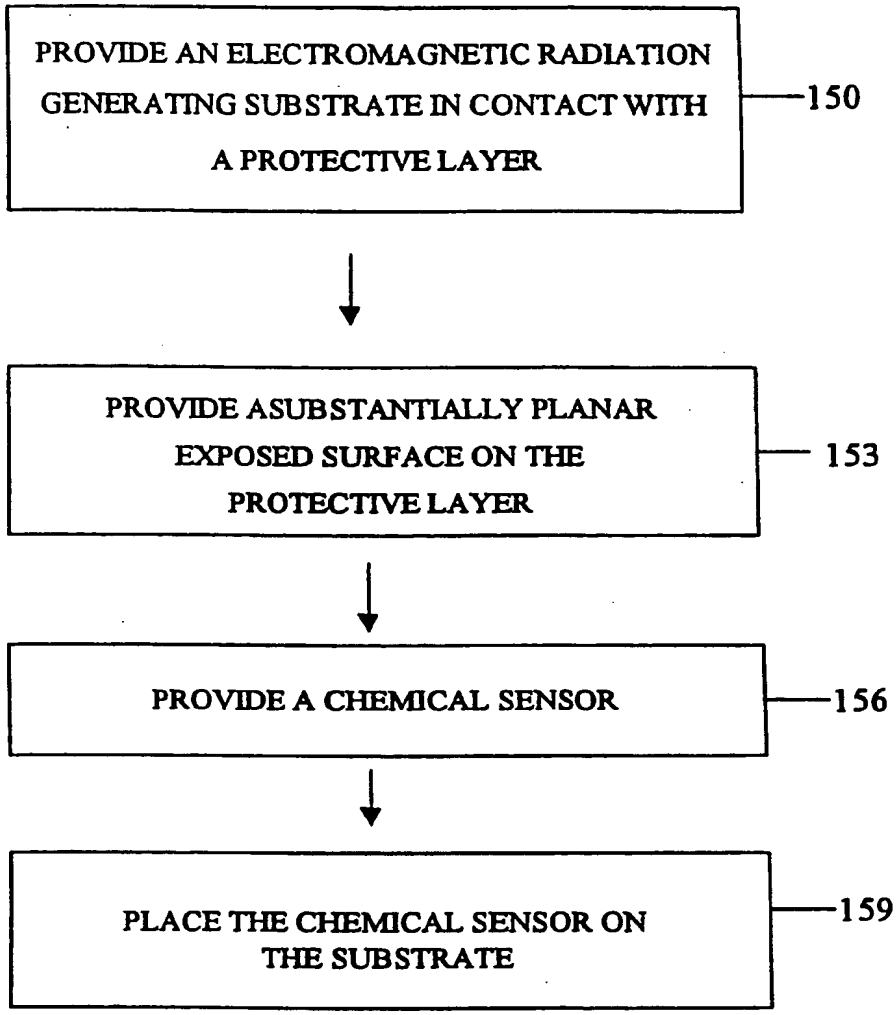

PROVIDE AN ELECTROMAGNETIC RADIATION GENERATING SUBSTRATE IN CONTACT WITH A PROTECTIVE LAYER
150
PROVIDE ASUBSTANTIALLY PLANAR EXPOSED SURFACE ON THE PROTECTIVE LAYER
153
PROVIDE A CHEMICAL SENSOR
156
PLACE THE CHEMICAL SENSOR ON THE SUBSTRATE
159

FIG. 8b

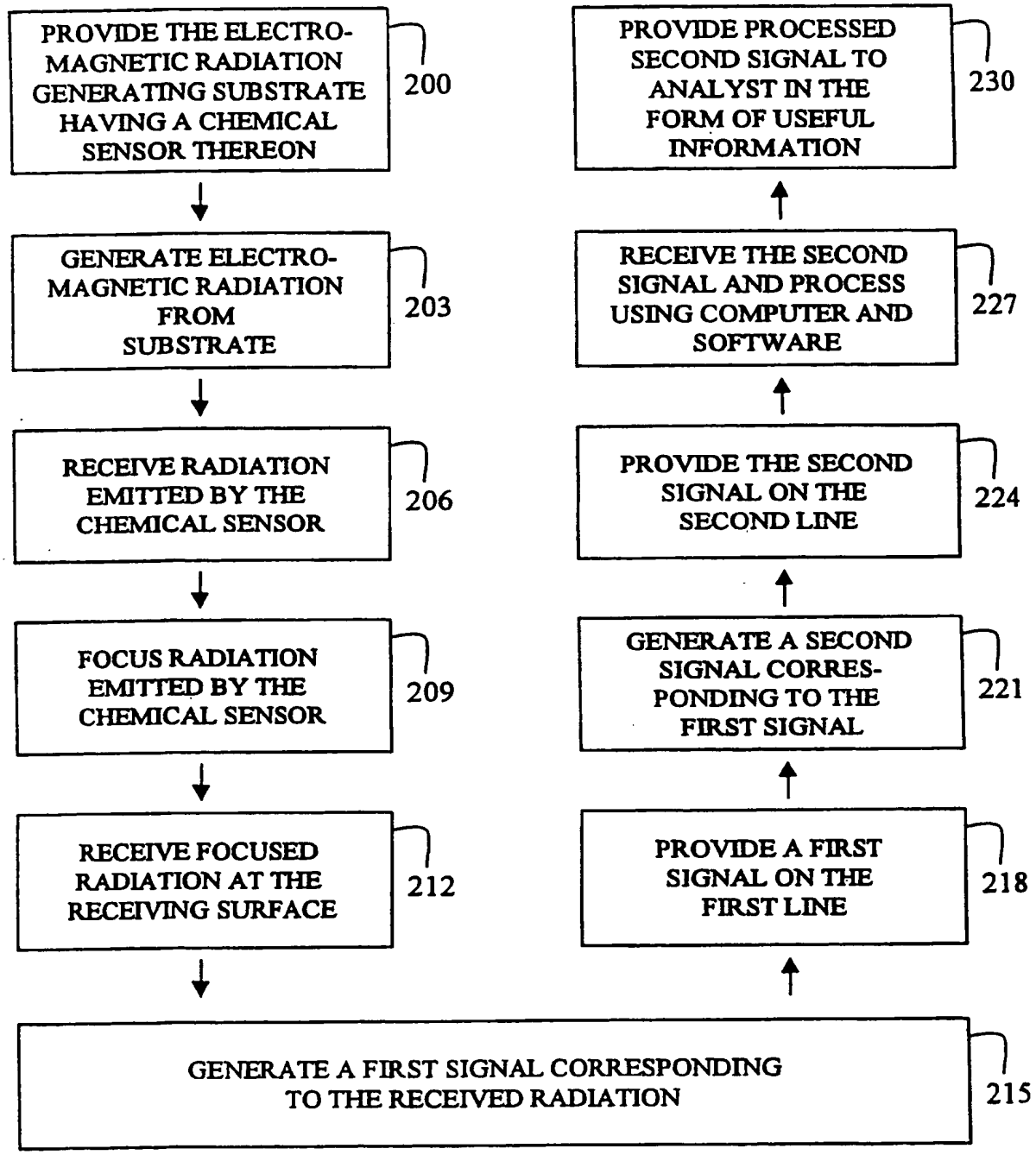

PROVIDE THE ELECTRO-MAGNETIC RADIATION GENERATING SUBSTRATE HAVING A CHEMICAL SENSOR THEREON
200
GENERATE ELECTRO-MAGNETIC RADIATION FROM SUBSTRATE
203
RECEIVE RADIATION EMITTED BY THE CHEMICAL SENSOR
206
FOCUS RADIATION EMITTED BY THE CHEMICAL SENSOR
209
RECEIVE FOCUSED RADIATION AT THE RECEIVING SURFACE
212
GENERATE A FIRST SIGNAL CORRESPONDING TO THE RECEIVED RADIATION
215
PROVIDE A FIRST SIGNAL ON THE FIRST LINE
218
GENERATE A SECOND SIGNAL CORRES-PONDING TO THE FIRST SIGNAL
221
PROVIDE THE SECOND SIGNAL ON THE SECOND LINE
224
RECEIVE THE SECOND SIGNAL AND PROCESS USING COMPUTER AND SOFTWARE
227
PROVIDE PROCESSED SECOND SIGNAL TO ANALYST IN THE FORM OF USEFUL INFORMATION
230

FIG. 9

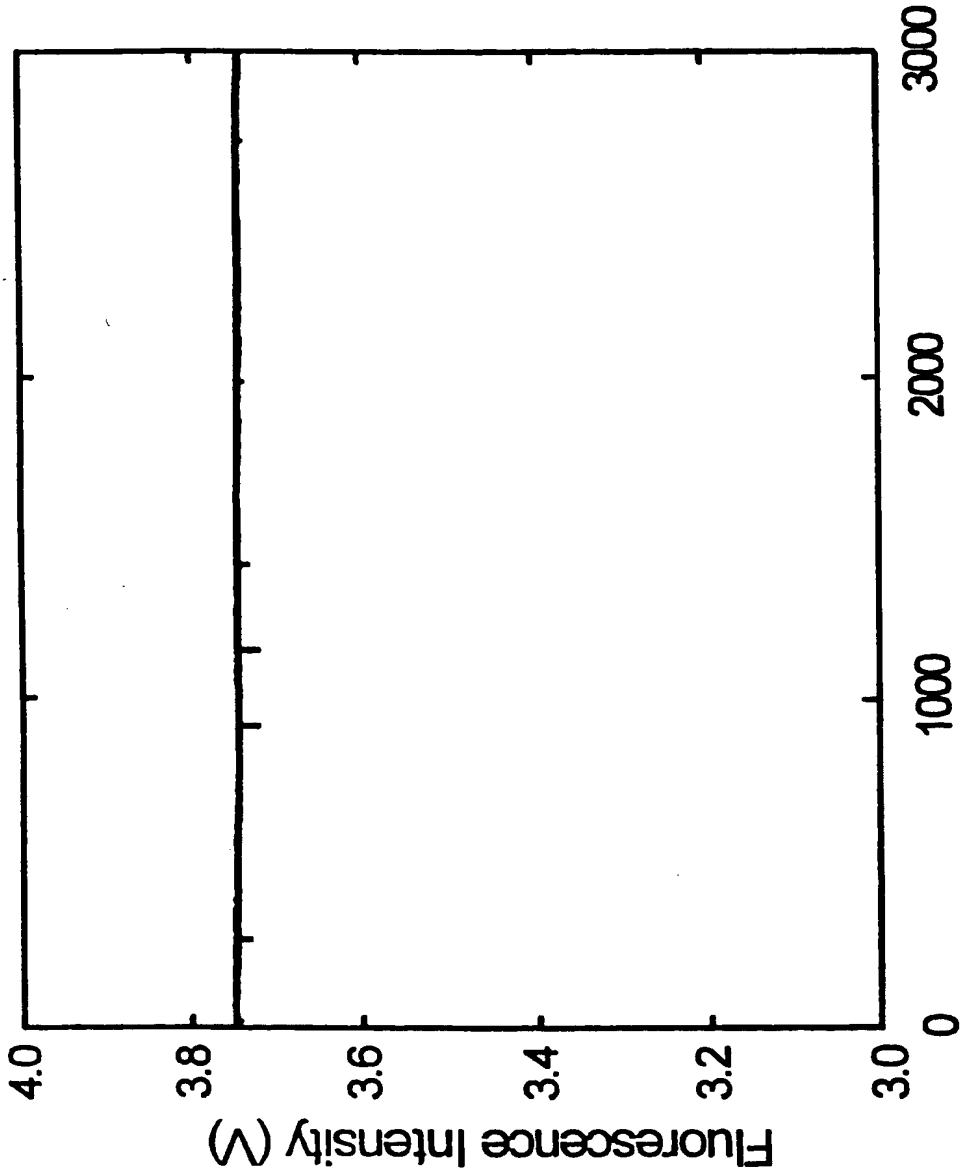

Fluorescence Intensity (V)
4.0
3.8
3.6
3.4
3.2
3.0
0
1000
2000
3000
Time (s)

FIG. 10

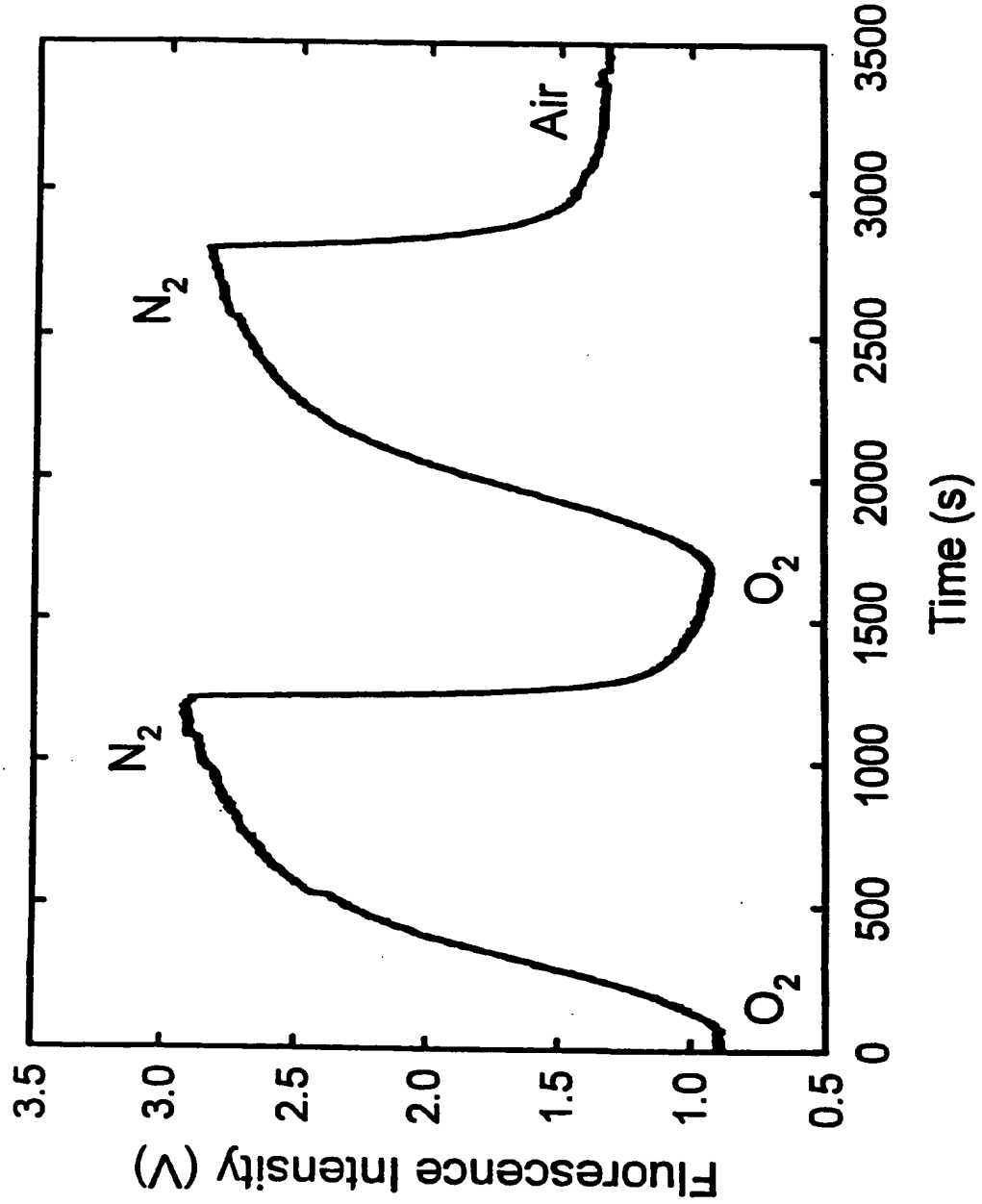

Fluorescence Intensity (V)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
500
1000
1500
2000
2500
3000
3500
Time (s)
O_2
N_2
O_2
N_2
Air

FIG. 11

FIG. 12a

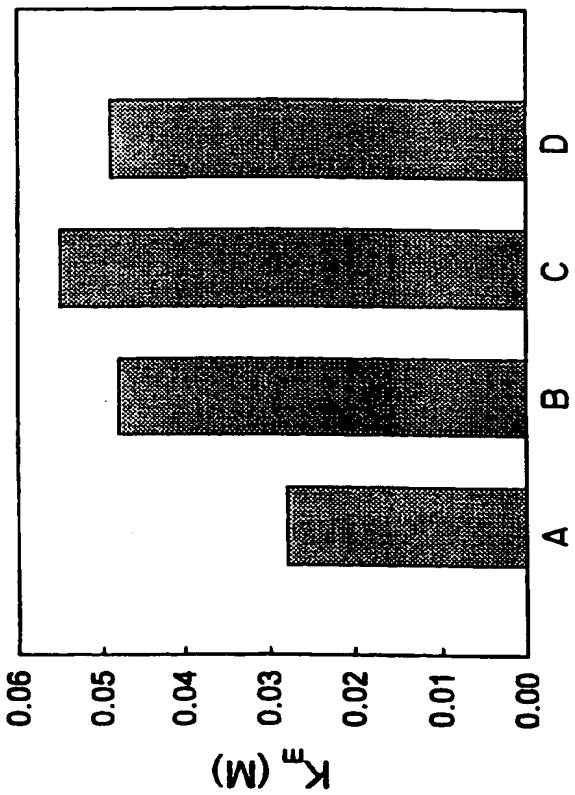
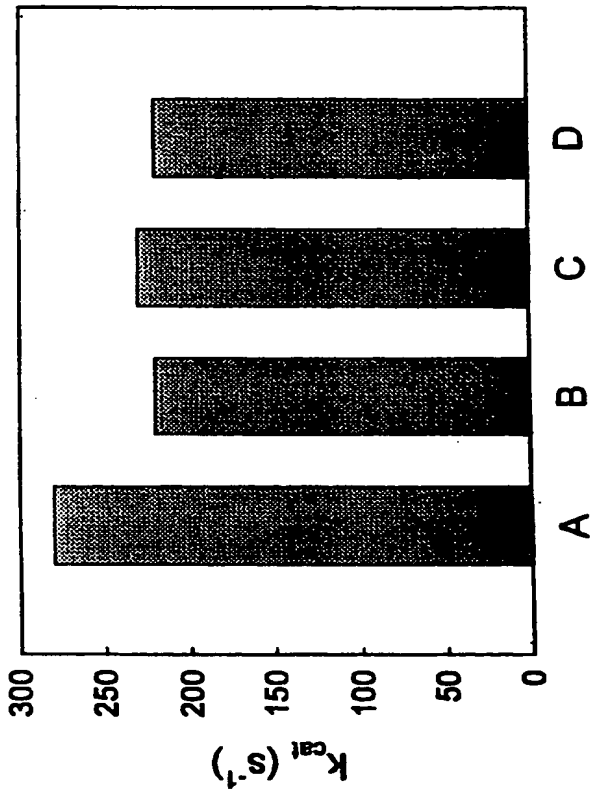


FIG. 12b



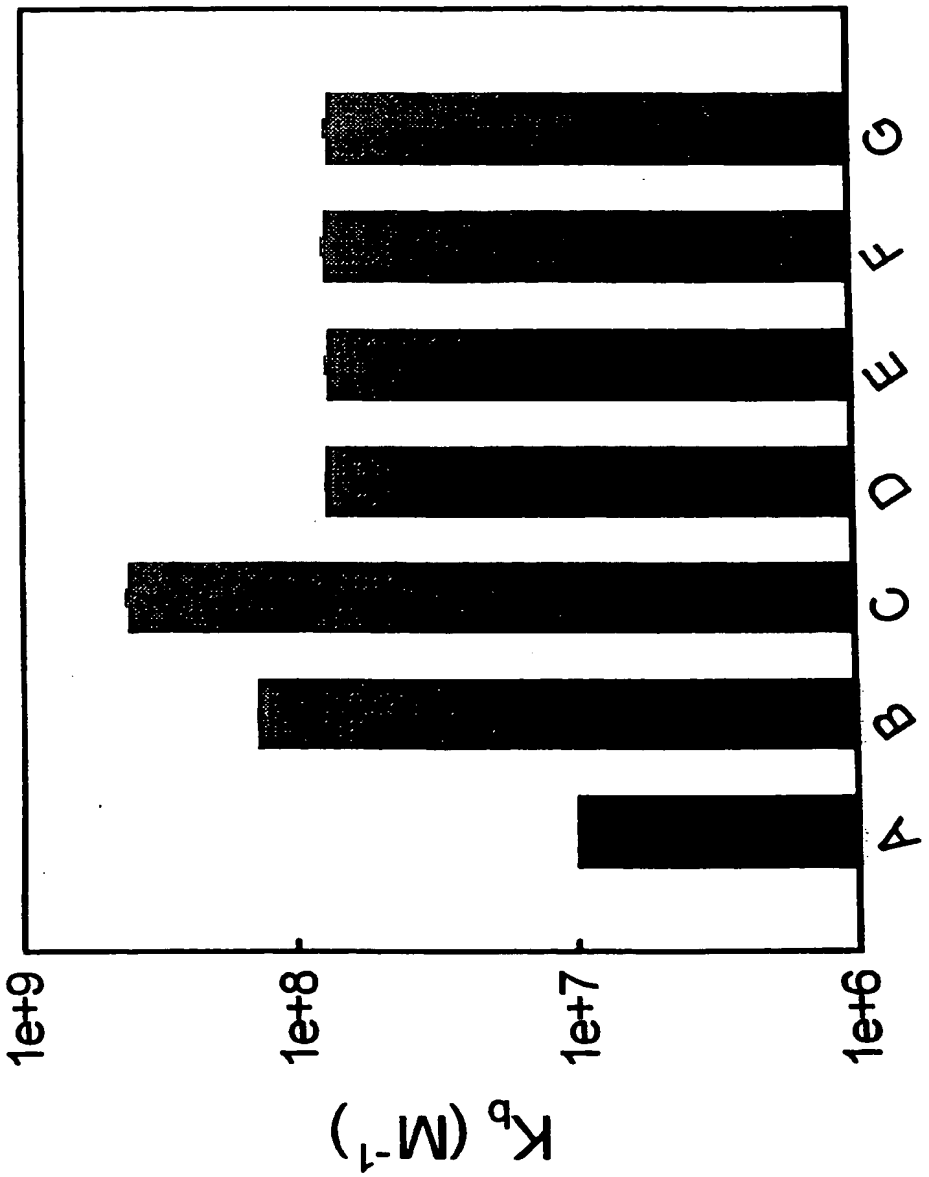


FIG. 13

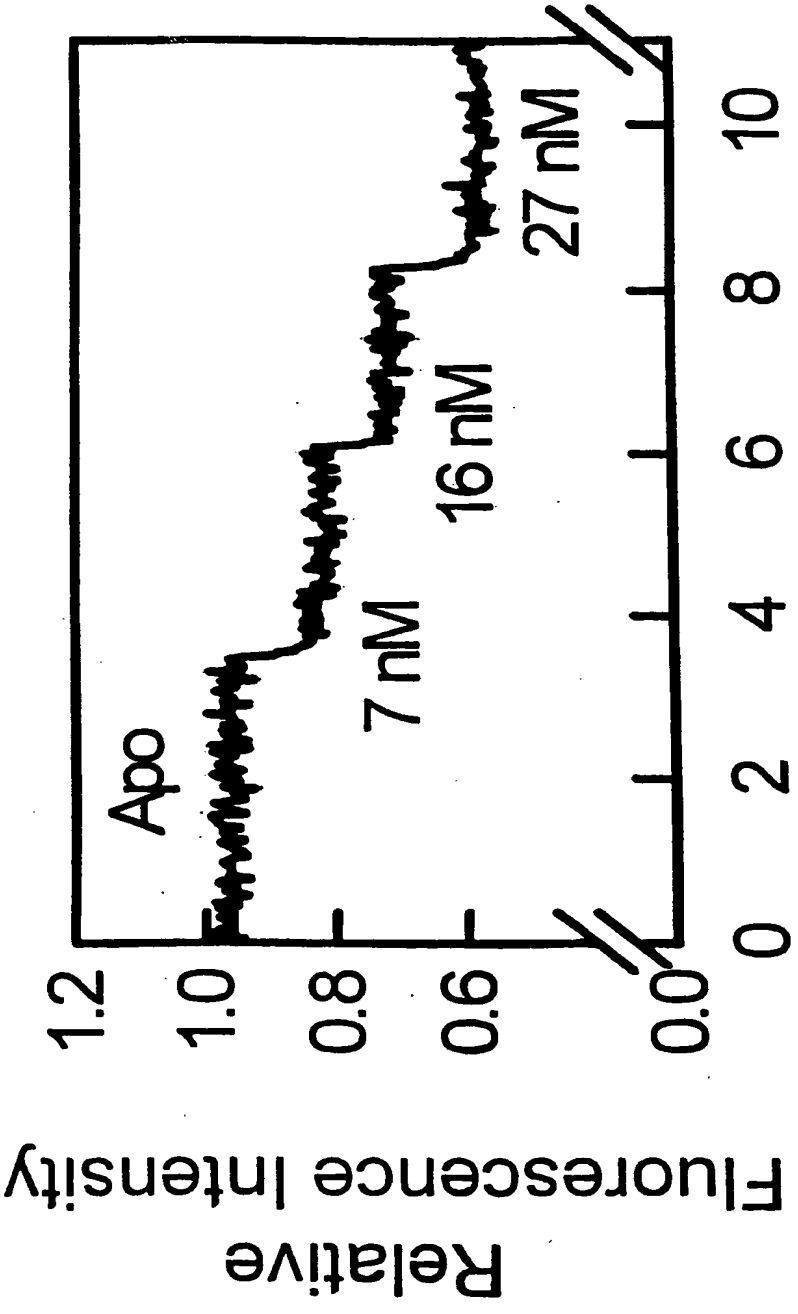
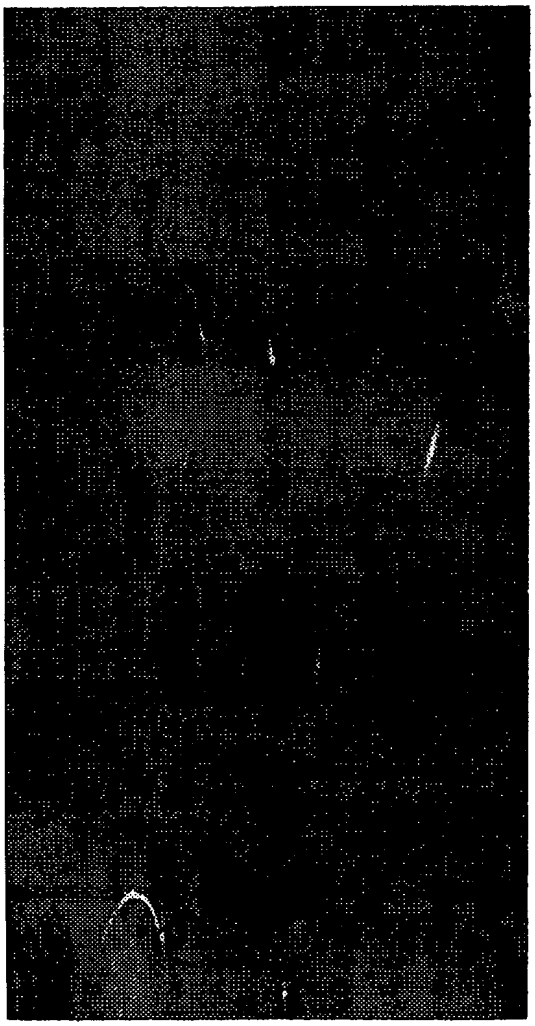

Relative
Fluorescence Intensity
1.2
1.0
0.8
0.6
0.0
0
2
4
6
8
10
Time(min)
Apo
7 nM
16 nM
27 nM

FIG. 14

FIG. 15a

FIG. 15b



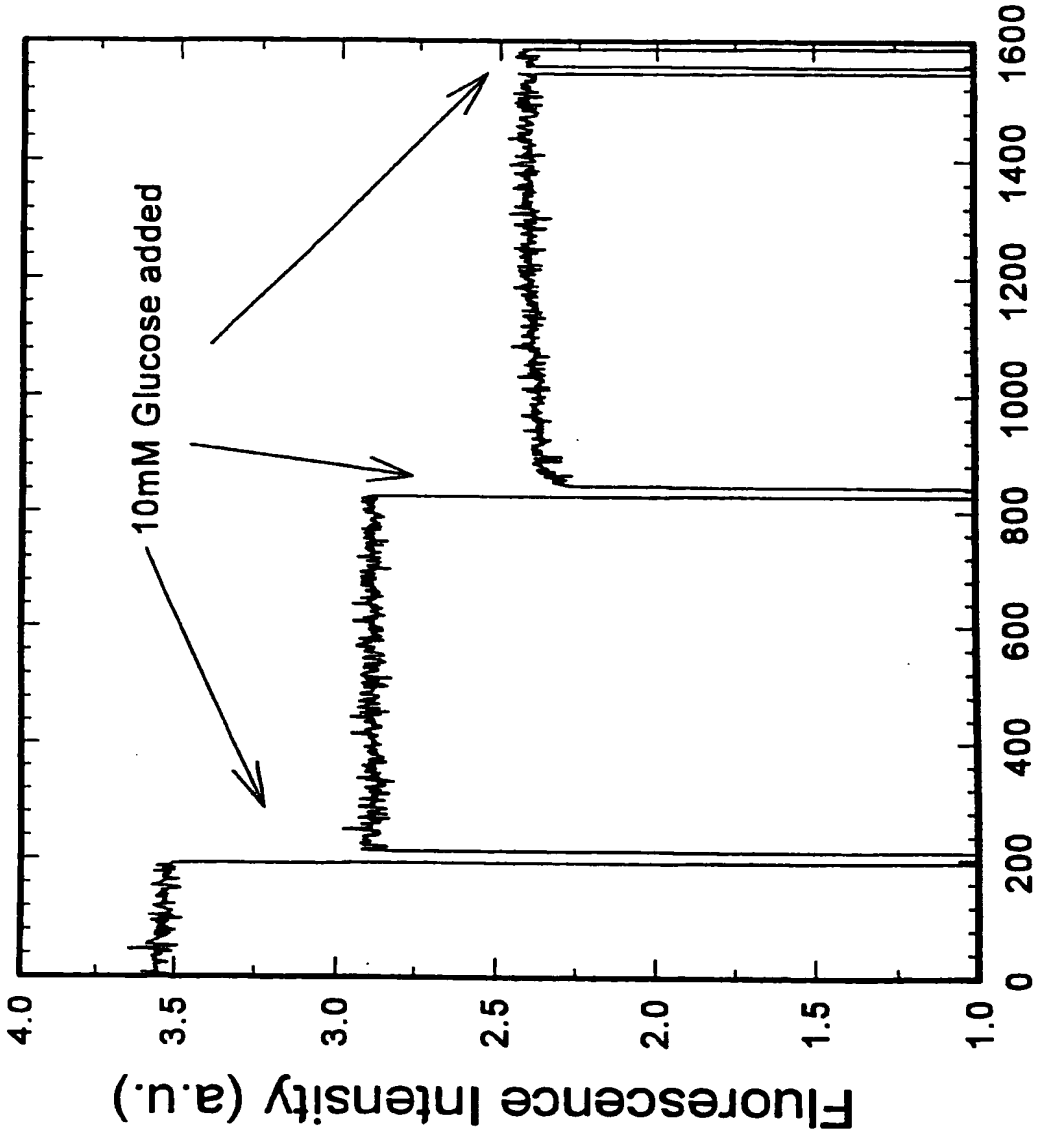

10mM Glucose added
Fluorescence Intensity (a.u.)
Time (sec)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0
200
400
600
800
1000
1200
1400
1600

FIG. 16

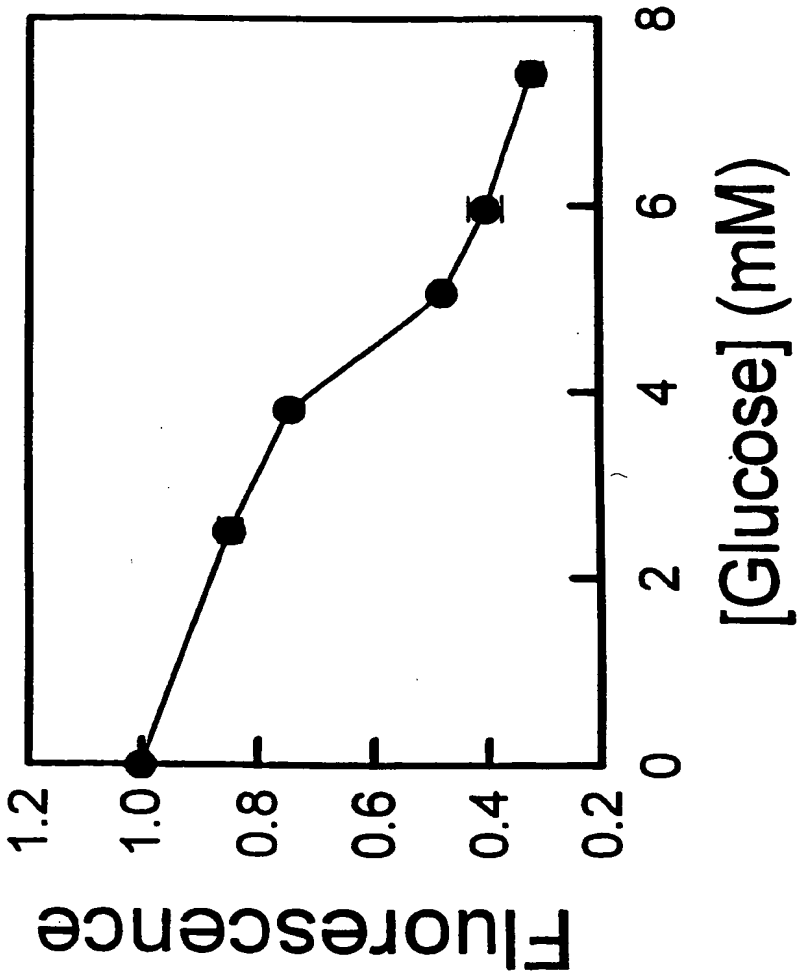


FIG. 17

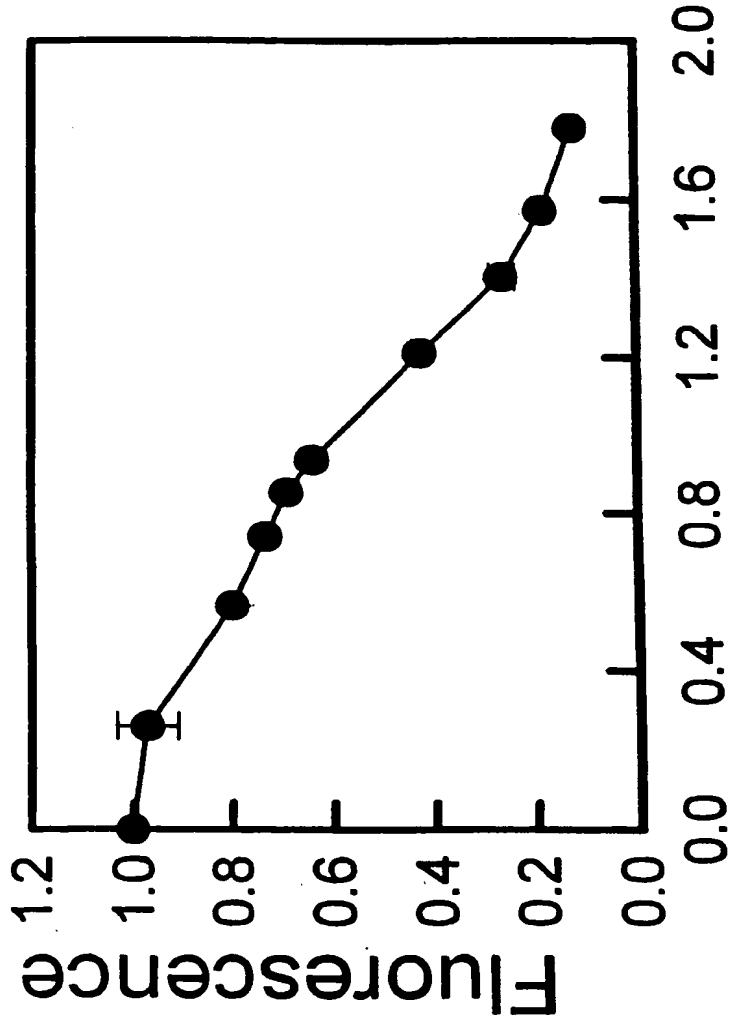


FIG. 18

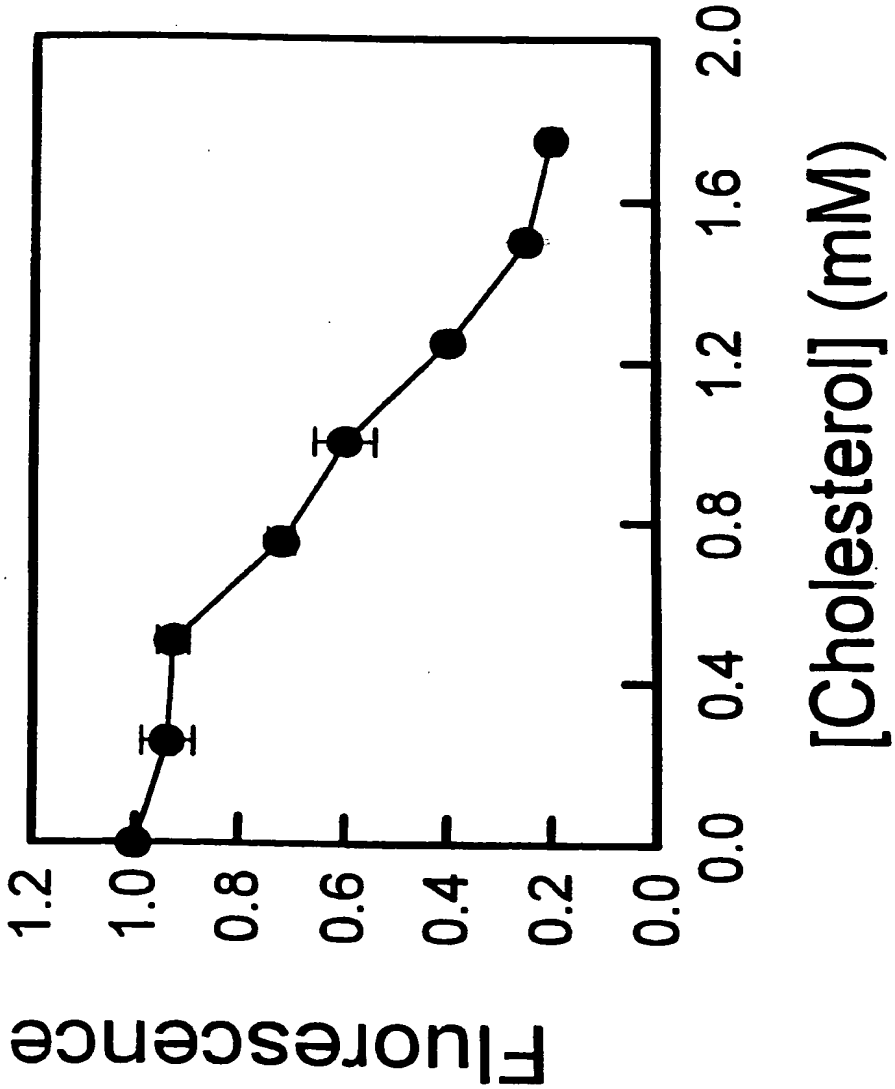

Fluorescence
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.4
0.8
1.2
1.6
2.0
[Cholesterol] (mM)

FIG. 19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/20646

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :G01N 33/48
US CL :422/82.05

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/82.05,56,58,61,68.1; 436/46,48

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NONE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,281,395 A (MARKART et al) 25 January 1994, see fig. 4 and col. 1	1-9,13-22,24-30,32-34 --- 10-12,23,31

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 26 OCTOBER 2000	Date of mailing of the international search report 14 NOV 2000
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Form PCT/ISA/210 (second sheet) (July 1998)*